You build me up, you break me down

van der Wijk, A.-E.

5

Is leukostasis a crucial step or epiphenomenon in the pathogenesis of diabetic retinopathy?

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ABSTRACT

Leukostasis in the retinal microvasculature in animal model studies of diabetes is associated with the development of diabetes-like retinopathy. Therefore, it is generally assumed that adhesion of leukocytes is a central event inciting a chronic, low-grade form of inflammation that causes the vascular abnormalities that are specific for the early stages of diabetic retinopathy (DR), which culminate in diabetic macular edema, proliferative DR, and vision loss in humans. Here, we review the literature critically with respect to leukostasis and assess its pathologic consequences in the human diabetic retina. First, we review the pathologic processes that are known to be involved in the development of human DR. Then, we summarize experimental evidence for the role of leukostasis in the development of DR and the mechanisms involved in leukostasis in the retina. Based on our critical review, we conclude that leukostasis may be an epiphenomenon of the diabetic retinal milieu, rather than a crucial, specific step in the development of human DR.

INTRODUCTION

DR is a leading cause of blindness in working-age individuals in developed countries [1]. The earliest clinical changes in the diabetic retina occur in the microvasculature and, as such, DR has traditionally been considered a vascular disease. Pericyte loss, thickening of the vascular basal lamina (or LB), breakdown of the BRB, and acellular capillaries are preclinical phenomena that are considered hallmarks of the early stages of DR [2]. As the disease progresses, saccular microaneurysms and hemorrhages appear and increasingly larger areas of nonperfused capillaries become evident. This nonperfusion, in combination with the loss of BRB, facilitates the formation of retinal exudates and retinal edema in the macula with loss of vision. In more-advanced disease, when the areas of nonperfused retina become large enough, neovascularization may develop, which leads to vitreal hemorrhaging, scarring, and retinal detachment with subsequent severe vision loss. Although each of these individual signs of DR may also be observed in other ischemic retinopathies, the usual constellation of these findings in the fundus of a patient with DR is so specific that no other diagnosis is possible. Therefore, DR is a unique disease of the retina, which only develops in patients with diabetes, and is initiated by hyperglycemia or other factors related to the diabetic milieu. It develops independently of other diabetic microvascular complications, but the local cause of the specific sensitivity of the retina to the diabetic milieu is still a matter of debate [3]. Therefore, the major questions in DR research are 1) how does diabetes cause the onset and progression of DR, and 2) why is the retina specifically sensitive to diabetes? Here, we review and discuss the possible role of leukostasis and associated low-grade inflammation in DR in the context of those questions.

LEUKOSTASIS IN ANIMAL MODELS OF THE DIABETIC RETINA

Leukostasis in situ

The phenomenon of leukostasis in the vasculature of retinas of diabetic rats was first described by Schröder et al. [4]. In their histochemical study of retinal whole mounts obtained from perfusion-fixed diabetic rats, significantly more capillary-occluding monocytes and granulocytes were observed as compared with control rats. These capillary-occluding leukocytes also displayed a strong spatial correlation with focal endothelial swelling, capillary loss, and formation of intraretinal microvascular abnormalities. Because the leukocytes were found to be activated in the diabetic rats and because of their known ability to induce cellular damage by releasing cytotoxic products, it was speculated that occluding leukocytes have a causal role in the pathogenesis of DR through the induction of direct damage to the endothelium and surrounding tissue [4].

Some 10 years later, strong support for that hypothesis was presented by Joussen et al. [5], who observed high numbers of propidium iodide–stained retinal endothelial cells, indicating increased numbers of dead or dying cells in mice with 11 mo of STZ-induced diabetes or 22 mo of galactosemia. This was not the case in nondiabetic controls

or in transgenic diabetic ICAM-1$^{-/-}$ and CD18$^{-/-}$ mice, in which less retinal leukostasis was observed. These transgenic mice also displayed reduced DR-associated retinal vascular pathology, including BRB breakdown, endothelial cell and pericyte loss, and acellular capillaries. The authors concluded that in long-term diabetes in rodents, a chronic, low-grade inflammation, initiated by leukostasis, induced the specific abnormalities of PCDR. These early abnormalities are considered to cause the initiation of more-advanced disease and vision loss in human DR and hence, the hypothesis that human DR is an inflammatory disease was born [6].

Recently, additional evidence for a causal role of leukocyte- induced vascular damage in PCDR in rodents was reported by various research groups, who demonstrated that after several intervention methods, decreased leukostasis led to decreased diabetic sequelae in diabetic animal models [5, 7–10], which will be further discussed in the "Experimental evidence for the role of leukostasis in the development of vascular pathology in DR" section

Leukostasis in vitro

In vitro studies using isolated retinal microvascular endothelial cells have been performed to further elucidate the specific effects of the hyperglycemic milieu on leukostasis. In one study, BRECs were exposed to high concentrations (20–100 mM) of D-glucose. This resulted in a dose-dependent increase in leukocyte adhesion. However, similar effects were obtained when BRECs were incubated in the presence of the same concentrations of mannitol instead of D-glucose, indicating that increased leukocyte adhesion was due to hyperosmolarity, rather than to the specific effect of excess D-glucose levels [11]. A similar experiment was performed with human retinal endothelial cells, which showed elevated leukocyte adhesion in the presence of high D-glucose concentrations (46 mM), whereas that effect was not observed when mannitol (30 mM) or L-glucose (30 mM) was used [12], suggesting that increased leukocyte adhesion to human retinal endothelial cells is a D-glucose–specific reaction and not a nonspecific reaction to a hyperosmotic environment. This interexperimental variation illustrates the difficulty of using in vitro models to study a complex systemic human disease, such as diabetes.

Leukostasis in vivo

Nishiwaki et al. [13] developed a technique to analyze leukocyte dynamics in the retinal microvasculature of laboratory animals in vivo. Leukocytes were labeled by an i.v. injection of acridine orange and were then visualized in the retinal microvasculature using a scanning laser ophthalmoscope. In various animal models, increased leukostasis in the retinal microvasculature was reported after short- and long-term diabetes using this technique [14–16]. On the other hand, in rhesus monkeys with spontaneous diabetes type 2 [17], static leukocytes in retinas with histologic evidence of DR were not different from those in retinas without signs of DR. Moreover, the db/db mouse, a model for diabetic dyslipidemia, did not show increased leukostasis in the retina [18].

Unfortunately, the toxic nature of acridine orange prevents the study of leukostasis in the retinal vasculature of humans with diabetes. A safe technique for visualizing leukocytes

in humans has yet to be developed. Therefore, the study of leukostasis in human retinal vasculature is not yet possible, and as a consequence, most of the current data on retinal leukostasis is derived from animal models of DR.

PROPOSED MECHANISMS OF LEUKOSTASIS

There are 3 mechanisms that have been proposed to lead to increased leukostasis in the retina during the development of DR: 1) decreased retinal blood flow or perfusion pressure; 2) narrowing of capillary lumina; and 3) increased leukocyte–endothelium adhesion.

Decreased retinal blood flow and perfusion pressure

The state of retinal blood flow in diabetes is controversial, with studies reporting both decreased and increased blood flow in the diabetic retina [19, 20]. One study of retinal hemodynamics showed that the number of static leukocytes increased in diabetic rats, whereas leukocyte passage time through retinal capillaries was similar to that in controls [15]. In contrast, Abiko et al. [14] showed that increased leukostasis in Zucker diabetic fatty rats was accompanied by reduced retinal blood flow. However, treatment of diabetic rats with the antioxidant α-lipoic acid prevented the occurrence of leukostasis but did not normalize retinal blood flow. Therefore, it seems unlikely that decreased retinal blood flow or perfusion pressure is the mechanism of retinal leukostasis.

Narrowing of capillaries

Luminal narrowing of retinal capillaries has been observed in diabetic OLETF rats [21] and primates with VEGF-induced retinopathy [22]. The exact mechanisms by which the narrowing occurs are largely unknown. In primates with VEGF-induced retinopathy, hypertrophy of the endothelial cells has been shown to cause lumen narrowing in retinal capillaries [22]. Vascular constriction has been proposed as a mechanism of leukostasis through capillary narrowing because expression of the vasoconstrictor ET-1 was found to be increased in the retinal vasculature of diabetic rats [23, 24], whereas the expression of ET-1 receptors on retinal pericytes was also found to be increased [24]. ET-1 receptor antagonists limited leukostasis in the retinas of rats with STZ-induced diabetes, but that effect was attributed to decreased VEGF production, rather than to vasoconstriction [25]. Therefore, the evidence is minimal at best that capillary constriction is a cause of leukostasis in the diabetic retina.

Vascular compression from LB thickening has also been suggested to cause lumen narrowing [26]. LB thickening is a well-established, histopathologic feature of DR [27, 28], but it has not been shown to cause lumen narrowing or leukostasis in retinal capillaries [29]. Furthermore, there is no spatial correlation between the vascular complications attributed to increased leukostasis, which occur nonuniformly throughout the retina of patients with diabetes, whereas LB thickening occurs uniformly, making LB thickening a less-likely cause of leukostasis [30].

Lumen narrowing has yet to be demonstrated in retinal capillaries of humans, but a number of findings support the feasibility that it causes leukostasis in humans. First, leukocytes isolated from patients with diabetes have decreased elasticity [31]. Because the diameter of most leukocytes is roughly twice that of the average retinal capillary, adequate elasticity is crucial for leukocyte passage. Second, vessels in the diabetic retina have been shown to be more tortuous compared with nondiabetic retinas in both rodents [21, 32] and humans [33]. Therefore, more-rigid leukocytes can become trapped at tortuous sites when blood flow is hampered in retinas of patients with diabetes. However, to date, no direct evidence exists for this mechanism of leukostasis in the human diabetic retina.

Increased leukocyte-endothelium adhesion

Considerably more evidence has been generated in both animal models and patients that implicate low-grade, chronic inflammation in inducing leukocyte–endothelial adhesion as a major mechanism of increased leukostasis in diabetic retinas [5]. Leukocytes isolated from diabetic rats and humans show increased adhesion to endothelial cells in vitro [34, 35]. Additionally, leukocytes from diabetic rats and patients express increased levels of the β_2-integrins CD11a, CD11b, and CD18 [34, 36]. These adhesion molecules, in conjunction with their endothelial counterparts ICAM-1 and VCAM-1, have a crucial role in inflammation because they are required for firm leukocyte–endothelial cell adhesion. Abs against CD11a, CD11b, and CD18 prevented adhesion in vitro, and CD18 blockade suppressed leukocyte adhesion in retinal capillaries of diabetic rats in vivo [34]. Furthermore, activated leukocytes that were injected into healthy mice were shown to interact with retinal endothelium in association with increased ICAM-1 staining [37]. That indicates that activated leukocytes have the capacity to locally upregulate endothelial ICAM-1 expression. Administration of anti–ICAM-1 Abs significantly reduced leukostasis in diabetic rats [38]. Moreover, leukostasis did not occur in ICAM-1– or CD18-deficient mice at 11 mo of STZ-induced diabetes or 22 mo of galactosemia [5].

In the diabetic human retina, the relevance of ICAM-1 expression has not yet been established. Both increased and unaltered ICAM-1 expression has been found [39, 40]. The different results of these 2 studies may be explained by the size of the control groups. One study used a small group of 6 control retinas, 4 of which were devoid of ICAM-1 staining, whereas 2 were not [40]. The other study included a control group equal in size to the diabetic groups that were studied (n = 19). In that study, most of the control retinas exhibited low to moderate ICAM-1 staining that was similar to that in diabetic retinas [39].

In agreement with the latter study, moderate, constitutive, endothelial ICAM-1 expression has been reported in human retinal capillaries in vivo [41] and in human retinal endothelial cells in vitro [42, 43]. Moreover, the adhesion molecules VCAM-1, E-selectin, and P-selectin, which are important in the inflammatory process through their tethering of leukocytes to the endothelium, have not been found expressed in the healthy or diabetic human retinal microvasculature [39, 40].

CURRENT PARADIGM OF LEUKOCYTE-INDUCED VASCULAR PATHOLOGY LEADING TO DR

In this section, we present a synopsis of data regarding the molecular processes that lead to leukostasis and PCDR, as observed in diabetic animal models. Furthermore, we attempt to incorporate those processes into a simplified chain of events that represents the theory of how leukostasis and low-grade inflammation lead to the specific vascular pathology of DR.

Many biologic factors and conditions have been shown to modulate retinal leukostasis. Tables 1 and 2 contain a comprehensive list of factors and conditions that either induce or reduce leukocyte adhesion in the retinal circulation in experimental models. The hyperglycemic state induced by diabetes has been proposed to lead to DR through 3 major pathologic biochemical processes: 1) increased flux of glucose metabolites through the polyol pathway [56], 2) nonenzymatic protein glycosylation and the resulting accumulation of AGEs [57, 58], and 3) increased oxidative stress from accumulation of ROS [43, 59, 60]. These latter 2 processes have also been implicated in increased retinal leukostasis. Systemic administration of AGEs leads directly to increased retinal leukostasis in mice [48], whereas various antioxidant therapies neutralize the diabetes-induced increase in leukostasis [14, 61].

Table 1. Factors known to increase retinal leukostasis

Factors increasing leukostasis	Fold increase*
Diabetes	1.9 [44]–4.8 [17]
Increased glucose	1.9 [12]
Hyperosmolarity	1.6 [11]
VEGF	4.8 [45]–14.5 [46]
TNF-α	13–92 [46, 47]
IL-1β	4.5 [46]
Platelet-activating factor	13.6 [46]
AGEs	2.7 [48]
Insulin	2.5 [49]
Hypertension	2–2.7 [50]
Hypercholesterolemia	2.1 [51]

*Fold increase relative to control experiments.

Hyperglycemia itself may also directly lead to increased leukostasis through PKC-β activation, likely via de novo synthesis of diacylglycerol [62]. AGEs and ROS also lead to activation of PKC-β [14, 63]. As such, it is conceivable that PKC-β activation is a common mechanism through which AGEs, ROS, and hyperglycemia lead to leukostasis and DR. Activation of PKC-β leads to increased transcription of various proteins and growth

factors, such as VEGF [64], which can lead to enhanced vascular permeability and retinal leukostasis and coincides with increased ICAM-1 expression [45]. In human DR, a PKC-β inhibitor was shown to moderately reduce visual loss, need for laser treatment, and macular edema progression in patients with diabetes [65]. In the rat retina, inhibition of PKC-β activation has been shown to ameliorate diabetes-induced leukostasis [14].

Leukocytes activated by the diabetic milieu in rodents and patients with diabetes adhere to the retinal vasculature and induce endothelial apoptosis [9, 43, 66] through Fas–Fas ligand interactions [66]. It has further been proposed that this chain of events possibly leads to vision loss via 2 separate pathways [66]. First, breakdown of the BRB can (at least in part) occur via induction of apoptosis in the endothelium. This endothelial cell death results in vascular leakage, which may contribute to the development of macular edema. Second, the accumulative effect of endothelial cell death because of the chronic nature of diabetes may lead to replicative senescence and avascular capillaries. That results in areas of retinal nonperfusion and hypoxia that induces increased VEGF expression, which ultimately leads to retinal neovascularization [66]. A schematic representation of those proposed pathways based on animal model studies is shown in Fig. 1. Despite the fact that the scheme is a simplified representation of the processes at hand, it provides an adequate overview of mechanisms of vascular pathology of DR in rats and mice, including leukostasis and inflammation.

Table 2. Factors known to decrease diabetes-induced retinal leukostasis

Factors decreasing leukostasis in DM	Fold decrease*
Aspirin	4.9 [52]
COX-2 inhibitor	5.6 [52]
Etanercept (anti–TNF-α)	5.1 [52]
Endothelin	2.1 [25]
Antioxidants	
α-lipoic acid	1.6 [14]
D-α-tocopherol	1.7 [14]
Corticosteroids	2.3 [53]
PKC-β inhibition	1.7 [14]
PPAR-γ signaling	1.6 [44]
5-Lipoxygenase deficiency	15 [54]
12/15-Lipoxygenase deficiency	6.5 [54]
AMA0428 (ρ-kinase inhibitor)	1.4 [7]
Vitamin B (Metanx)	1.7 [55]

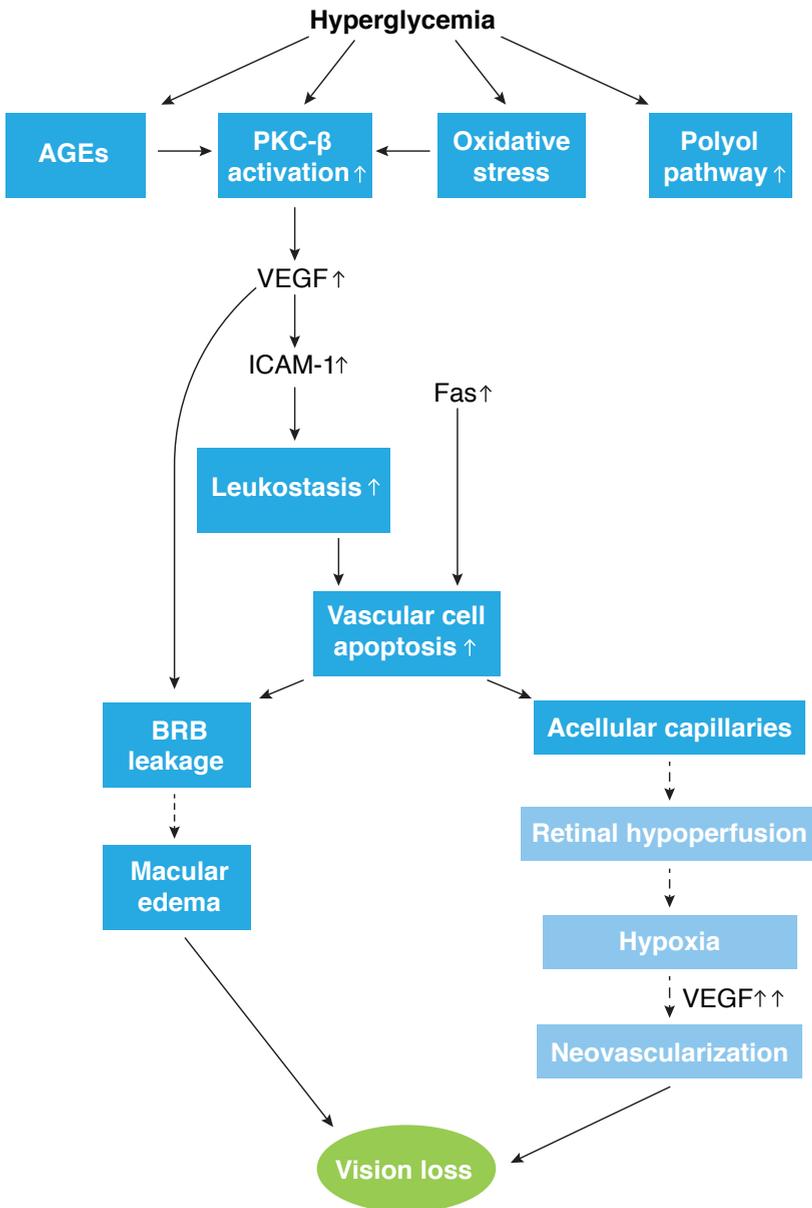***Fold decrease relative to diabetic controls with vehicle only.**

Figure 1. Schematic diagram of the role of leukostasis in the development of DR in animal models based on current literature. Pathways represented by dashed arrows are based on conjecture, whereas solid arrows represent pathways that have been shown to be involved.

Table 3. Comparison of retinal lesions found in various well-characterized animal models of DR, hypertension and hyperlipidemia as well as in human DR

	Pericyte loss	Acellular capillaries	Micro-aneurysms	LB thickening	Capillary narrowing	BRB leakage	Capillary tortuosity/loop formation	Exudates	Haemorrhages	Capillary non-perfusion	Cotton wool spots	IRMA	Neovascularisation
Human diabetes (2)	+	+	+	+	+	+	+	+	+	+	+	+	+
Spontaneous diabetic rhesus monkeys (17, 86) (15 years)	-	+	+	+				+	+	+	+	+	-
Alloxan-induced diabetic dogs (87) (5 years)	+	+	+	+				+	+			+	-
Galactose-fed dog (88) (5 years)	+	+	+	+				+	+	+		+	+
Galactose-fed rats (89) (23 months)	+	+	-	+					-		+	+	-
Sucrose-fed diabetic Cohen rats (90) (26 weeks)	+	+	+	+						+			-
STZ-induced diabetic rats (91, 92)(12 months)	+	+	+	+	+	+							-
Diabetic BB rat (93) (4 months)	-	-	-	-		-							-
OLETF rats (21) (14 months)	-	-	+	+	+		+	-	-				-
Zucker diabetic fatty rat (94, 95) (5 months)	-	-	-	+	-		-		-				-
Spontaneous diabetic Torii rat (96, 97) (60 weeks)	-	-	-	+		-	+	-	+	+			+
Galactose-fed mice (5) (24 months)	+	+	-	+		+							
STZ-induced diabetic mice (98) (18 months)	+	+	-	-									-
db/db diabetic mice (18) (10 weeks)	+	+	-	+	-	-							
RICO rats (99) (18 months)	+	+		+	+	+	+						-
SHR rats (92, 100) (7 months)	+	+	+	+	+	+	+			+			

EXPERIMENTAL EVIDENCE FOR THE ROLE OF LEUKO-STASIS IN THE DEVELOPMENT OF VASCULAR PATHOLOGY IN DR

The mounting evidence that leukostasis has a role in the development of sequelae characteristic for PCDR in diabetic animal models is the major basis of the assumption that human DR is a disease caused by low-grade, chronic inflammation [6]. This idea is not new (DR was initially termed "diabetic retinitis" [67]), but the data implicating increased retinal microvascular leukostasis as having a specific causal role in the development of PCDR has provided new support for the chronic inflammation hypothesis. The evidence seems substantial that this is the case in animal models, but a critical analysis of the current literature reveals conflicting evidence of this causal role.

TNF-α–dependent leukostasis was shown to be responsible for increased endothelial cell death and BRB leakage in rat retinas as early as 1 wk after induction of diabetes [52]. The TNF-α–dependent nature of VEGF-induced leukostasis was demonstrated in TNF-α knockout mice. However, BRB leakage was not significantly altered in that model, and the absence of TNF-α and leukostasis did not prevent or reduce retinal neovascularization when those mice were used in an oxygen-induced retinopathy assay [46]. Taken together, these data suggest that leukostasis may not be essential in animal models for the development of PCDR.

Leukostasis did not occur in ICAM-1$^{-/-}$ and CD18$^{-/-}$ mice with STZ-induced type 1 diabetes up to 11 mo. Retinas of those mice exhibited significantly fewer acellular capillaries and no altered numbers of pericytes and endothelial cells as compared with wild type diabetic mice [5]. STZ-induced diabetic, transgenic mice expressing neutrophil inhibitory factor, which is a selective antagonist for the integrin complex Mac-1 (CD11b/ CD18), do not develop retinal leukostasis, and in those mice degeneration of retinal capillaries and retinal superoxide production were inhibited [9]. In addition, recent studies demonstrated that inhibition of ROCK signaling [7], VEGFR1 blockade [68], retinal overexpression of ACE-2 [69], or inhibition of the VEGF coreceptor neuropilin-1 [70] attenuated leukostasis in the retina of mice with STZ-induced diabetes, and that was accompanied with decreased diabetes-induced retinal complications. These data suggest that leukostasis is causal for the development of sequelae characteristic for PCDR and non-proliferative DR.

In contrast, several recent studies provide evidence that this causal association is not always the case. Spontaneously diabetic db/db mice, known to develop acellular capillaries at 34 wk of age, showed no increase in leukostasis, suggesting that other factors than leukostasis lead to acellular capillaries in this model of type 2 diabetes [18]. Both STZ-induced diabetic mice deficient in either 5-lipoxygenase or 12/15-lipoxygenase, which are inducers of chronic inflammation and ROS production, demonstrated a similar reduction in leukostasis. However, pericyte loss and numbers of acellular capillaries were reduced only in the 5-lipoxygenase–deficient diabetic mice, whereas in the 12/15-lipoxygenase–deficient diabetic mice, those sequelae developed in spite of the reduction in leukostasis [54]. Moreover, when STZ-induced diabetic mice were treated with the medical food product

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Metanx (containing the active forms of vitamins B_9, B_6, and B_{12}; Alfasigma, Covington, LA, USA), multiple inflammation-related molecular abnormalities, such as leukostasis, ICAM-1 expression, and activation of NF-κb in the retina, were significantly inhibited, but no effects were observed on degeneration of retinal capillaries and formation of pericyte ghosts [55].

In our opinion, these studies provide evidence that dissociates the occurrence of leukostasis and low-grade inflammation from the development of the specific diabetic retinal sequelae. Reasons for these contradictory results can possibly be found in differences in methodologies and diabetic models that have been used (toxin-induced type 1 diabetes vs. genetically inbred strains of spontaneously induced, type 2 diabetes) or perhaps in the genetic differences among species. This is exemplified by the variation in retinal pathology observed in the various DR models; all of which fail to replicate the entire spectrum of retinal sequelae seen in human DR (Table 3).

RELEVANCE OF LEUKOSTASIS IN THE DEVELOPMENT OF DR IN HUMAN DIABETES

In this section, we set out to assess the relevance of leukostasis in the development of human DR. In addition to differences in sizes and functions of ocular tissues between species, differences in vascular patterns [86], cellular composition [87], cellular metabolism, and biochemistry [88] have been demonstrated.

Furthermore, a discrepancy in NF-κb expression and activation exists between STZ-induced diabetes in rats and human patients with diabetes. NF-κb is exclusively expressed in retinal vascular pericytes of human patients with diabetes [89], whereas in diabetic rats, its activity is increased in both retinal endothelial cells and pericytes [90]. This is a crucial difference because NF-κB has an essential role in inflammatory processes, including leukostasis. Moreover, NF-κB is also involved in retinal pathology in experimental rodent diabetes through induction of various molecules, such as ICAM-1 and Fas in retinal vascular endothelial cells [66, 52, 55, 91], whereas increased vascular expression of either ICAM-1 [39] or Fas [unpublished data] was not found in human diabetic retinas.

The ocular differences between man and rodent are further illustrated by the various interventions that effectively decrease leukostasis and its associated sequelae in rodents but fail to be effective in preventing or slowing down progression of DR in humans. Treatment of diabetic rats with antioxidants reduces retinal leukostasis as well as the formation of pericyte ghosts and acellular capillaries [14, 61, 92]. Although properly randomized clinical trials involving therapeutic doses of antioxidants have yet to be performed, dietary antioxidant intake in human subjects with diabetes is not associated with a decreased incidence of DR [93]. Aspirin therapy reduces the formation of diabetic retinal sequelae in dogs [94] and rats [95] but has not proven to be effective in slowing down the progress of DR in humans [96].

Inhibition of PKC-β has also led to significantly decreased diabetes-induced retinal vasculopathies, including leukostasis in animal models [14], but the preventive effect on the incidence or progression of DR in humans is very small. Finally, intravitreal corticosteroid

injections seem to have similar effects in animal models and human diabetes because leukostasis and vascular leakage are decreased in diabetic rats [16], and diabetic macular edema and visual acuity in humans are improved [96], which is in agreement with the hypothesis that DR is a disease of chronic inflammation. However, corticosteroids may also have a substantial direct effect on BRB endothelium, independent of their anti-inflammatory actions [97].

Thus far, the only therapies proven to decrease the incidence and progression of DR in humans is strict control of hypertension and glucose levels in the blood using insulin [98, 99] or sulphonylureas [98]. Surprisingly, s.c. or ocular administration of insulin in rats leads to increased retinal leukostasis [49] and vascular pathology [100], respectively. This, however, is likely due to the direct effects of insulin on its many receptors in the retina, whereas the beneficial effects of systemically administered insulin in humans with diabetes are derived from its ability to lower blood glucose levels. Studies on the effect of systemic insulin therapy on leukostasis in animal models of diabetes have not yet been performed.

Interpretation of animal DR data with respect to human DR is difficult for at least two reasons. First, the reported outcome measurements vary. In animal studies, those measurements usually consist of quantitative biochemical data or qualitative pathology, whereas in human DR studies, the outcomes are mainly based on vascular macropathology and vision loss, neither of which occurs in current animal models of DR. Second, quantification of leukostasis in the human retina in vivo remains problematic. Therefore, the exact role of leukostasis in human DR remains speculative.

CONCLUSION: IS LEUKOSTASIS A MAIN PLAYER IN DR DEVELOPMENT OR AN EPIPHENOMENON?

Leukostasis and associated signs of low-grade inflammation are increased in most rodent models of DR. The evidence that these phenomena have a specific causal role in the pathogenesis of DR in these models is conflicting. As shown in Table 1, various factors lead to increased leukostasis in the rodent retina. However, not all of those factors lead to the sequelae characteristic for PCDR or nonproliferative DR, such as pericyte loss and acellular capillaries. Retinal leukostasis is also observed in rodent models of hypertension [50], insulin resistance [14], hypercholesterolemia [51], and hyperinsulinemia [49]. Those disease states also lead to various forms of vasculopathy in both the rodent and human retina, but none of them result in macular edema and vascular proliferation, causal for vision loss associated with DR. Breakdown of the BRB and vascular hypoperfusion are considered to precede those events. Leukostasis likely contributes to retinal vascular leakage, but it is not necessary for it to occur [46]. Furthermore, increased leukostasis does not reduce overall retinal blood flow [14]. Locally decreased blood flow induced by leukocyte capillary plugging might cause the development of acellular capillaries and regional hypoperfusion, but then, it would be expected to happen in the retinas of rodents with hypertension, hypercholesterolemia, and hyperinsulinemia, as well, which is not the case. Leukostasis can induce apoptosis of microvascular endothelial cells [9, 43, 66] and

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thus result in acellular capillaries and hypoperfusion. However, those sequelae are also observed in diabetic rodents without leukostasis [18]. Finally, when leukostasis is absent in diabetic retinas, the specific hallmark sequelae of PCDR or nonproliferative DR still occurs to the same degree [54, 55].

Taken together, it must be concluded that there is no compelling evidence for a significant role for leukostasis in the development of DR in humans. Increased leukostasis seems to be a result of aspecific endothelial cell dysfunction, rather than a crucial, specific step in the development of DR and is, therefore, likely an epiphenomenon of the retinal diabetic milieu. However, it cannot be ruled out (and is perhaps likely) that leukostasis enhances the pathogenic effects of the diabetic milieu on the retina. More research is needed to further elucidate the relevance of leukostasis and low-grade inflammation in the development of DR in the human retina. Improved animal models for DR, detailed pathologic studies in human DR, and the ability to quantify in vivo leukocyte dynamics in the human retina are critical to this endeavor.

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AUTHORSHIP

J.M.H. designed the content of the review and prepared the figures. A.-E.v.d.W. and J.M.H. wrote the manuscript. I.K., C.J.F.V.N., and R.O.S. advised in the design of the content of the review and contributed to editing of the manuscript.

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